Detection of PD-L1 in CTC from patients with bladder cancer using CytoGen's liquid biopsy platform

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ABSTRACT

Anti PD-1 immunotherapy has greatly enhanced the clinical outcomes of patients with late-stage metastatic bladder cancer. Accordingly, researchers try to estimate the relevance between the levels of PD-L1 and subsequent clinical outcome. However, the levels of PD-L1 expression highly fluctuate among different tissue biopsies, so this fluctuation and one-time tissue biopsy make it hard to predict prognosis in the bladder cancer patients treated with anti PD-1 immunotherapy. In an attempt to compensate for the problems in the tissue biopsies, we addressed the same question in a single cell level, i.e., circulating cancer cells (CTCs) from patients of the bladder cancer.

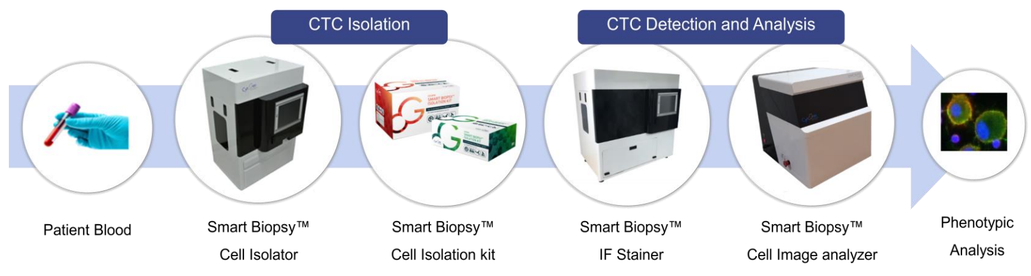
To address it, we isolated CTCs and examined the levels of PD-L1. The CTCs were isolated using CytoGen's Smart Biopsy Cell Isolator, a size-based gravity filtering system. Following the enrichment, the identities of isolated cells were assessed with immunocytochemical staining using three antibodies against EpCAM, Vimentin, and Cytokeratin as well as CD45 using CytoGen's SMART Biopsy IF Stainer. Then we defined CTCs showing the positive signal to those three epithelial markers and a negative signal to CD45. Then, we evaluated the level of PD-L1 expression in CTCs isolated from 25 patients with bladder cancer.

In our system, we succeeded in the detection of PD-L1 in isolated CTCs. These results showed that PD-L1 expression could be done with the CTC-based platform. The establishment of PD-L1 expression based on CytoGen's CTC platform is expected to provide more benefits to the patients who receive anti PD-1 immunotherapy in diverse cancers including bladder cancer.

METHODS

♦ CTC isolation and validation by CytoGen's Smart Biopsy System

CTCs were isolated from whole blood (5 ml) derived from patients with bladder cancer using Smart Biopsy Isolator (Fig. 1). The enriched CTCs were then validated by immunostaining using IF Stainer and analyzed by Cell Image Analyzer.



- ✓Automation system from CTC isolation to reporting.
- ✓Capture of Live CTCs.
- ✓Expression levels of the target proteins in CTCs
- ✓Size-based filtration (additional negative selection).
- ✓Quadruple IF staining & cell image analysis.

Fig. 1. Work flow of Smart Biopsy System for CTC isolation and validation

♦ Validation of CTC by immunostaining

CTCs were immunostained with epithelial markers, CK, EpCAM or mesenchymal marker Vimentin (VIM) using Smart Biopsy IF Stainer. CTC enumeration was done by Smart Biopsy Cell Image analyzer, based on expression of epithelial markers, CK, EpCAM or mesenchymal marker Vimentin (VIM), excluding CD45+ cells (Fig. 2). A portion of cells were all negative to CD45, CK, EpCAM and VIM, but positive to PD-L1. Since CK-, PD-L1+, CD45- cells were morphologically consistent with tumor cells and were counted to CTCs in previous study (4), we also counted those cells to CTCs.

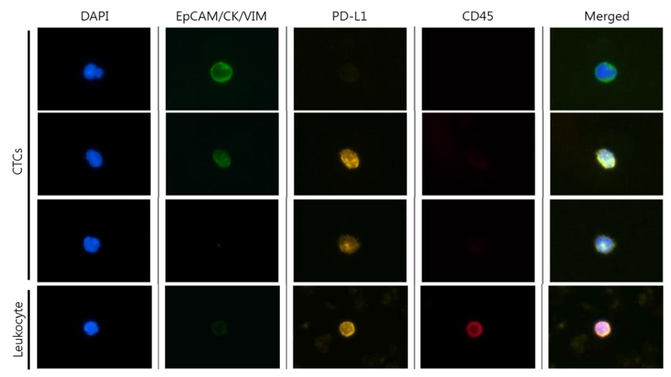


Fig. 2. Representative images of CTC isolated by Smart Biopsy System.

RESULTS

1. Validation of Smart Biopsy System for CTC analysis

For validation of Smart Biopsy System in CTC analysis, we tested the recovery efficiency using GFP-labelled H538 cancer cells spiked into whole blood with a known different -number of cells (100, 50 and 10 cells). Significantly, around 80% of the spiked cancer cells were recovered in all experiments (Table 1.)

Table 1. Spiking test with GFP-labelled H538 cancer cells

#H358-GFP cells spiked into 5ml whole blood	#Experiments	Mean (#cells retrieval)	S.T.D	Mean % retrieval
100	10	79	3.9	79%
50	10	41	4.3	82%
10	10	7.8	5.7	78%

2. Detection of PD-L1 in CTCs derived from the blood of patients with bladder cancer

For positive control, we chose H1975 lung cancer cell line due to its higher expression of PD-L1, compared with leukocytes in PBMC and other A549 cancer cells (Fig. 2A). CTCs were enriched from whole blood of patients with bladder cancer (n=25). Representative images are shown in Fig. 2C, in which PD-L1 levels varies in CTCs among patients.

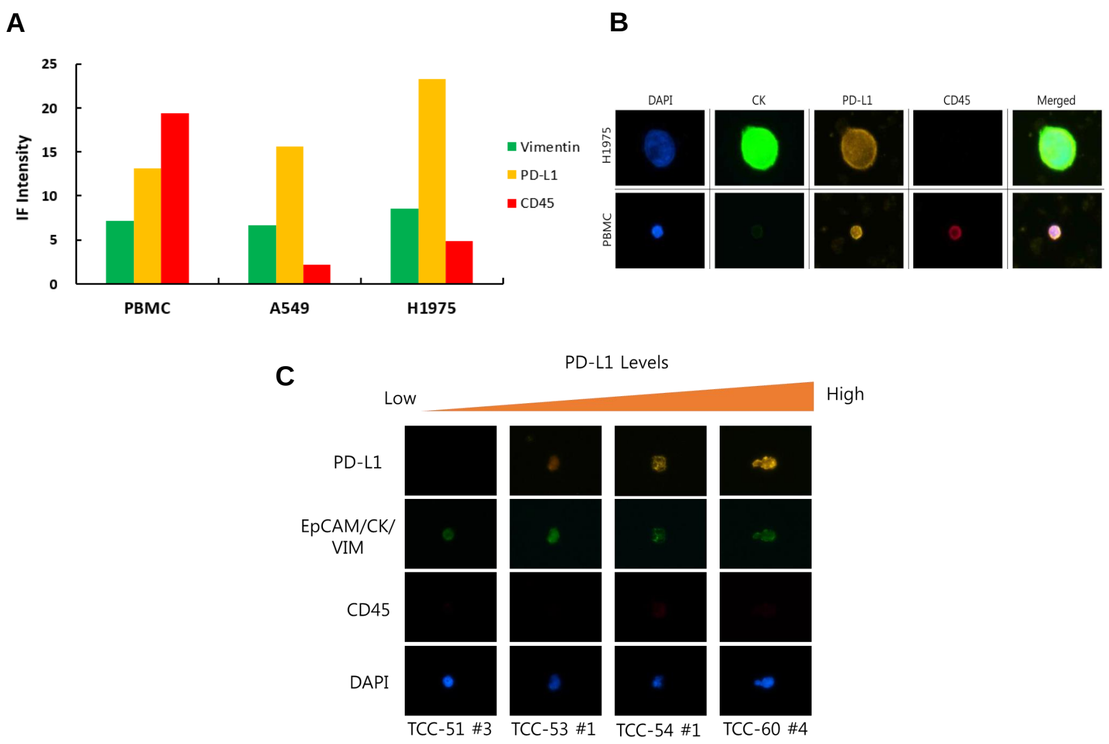


Fig. 2. Representative images of CTCs expressing PD-L1 at differential levels among patients with bladder cancer

3. Quantification of PD-L1 levels in CTCs derived from patients with bladder cancer

We analyzed CTCs of 25 patients with bladder cancer. CTCs were enumerated from 5 ml whole blood of patients with bladder cancer (Fig. 3A). The average number of CTCs was 3±0.47 in 25 patients. Among them, CTCs were not detected in two patients (#49, #52). Using Smart Biopsy Cell Image analyzer, PD-L1 levels in CTCs were quantified by immunofluorescence intensity (Fig. 3B). Patients were divided into three groups according to PD-L1 levels in CTCs, High (n=6), Intermediate (n=7) and Low (n=9). In further study, we will match PD-L1 levels in CTCs with the counterpart tissue biopsy and the clinical response of patients to immunotherapy. Taken together, these results suggest that PD-L1 levels could be quantified in CTCs using Smart Biopsy System.

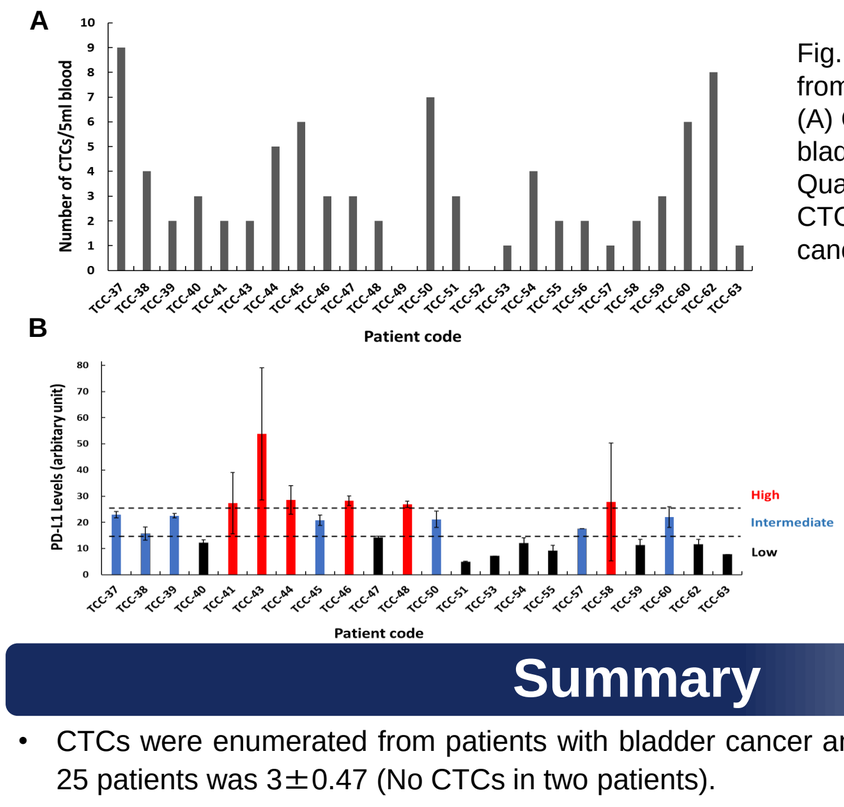


Fig. 3. PD-L1 levels in CTCs derived from patients with bladder cancer. (A) CTC enumeration in patients with bladder cancer (n=25). (B) Quantification of PD-L1 levels in CTCs of patients with bladder cancer.

Summary

- CTCs were enumerated from patients with bladder cancer and the average CTC number of 25 patients was 3±0.47 (No CTCs in two patients).
- PD-L1 levels in CTCs were quantified by immunofluorescence intensity. Patients with bladder cancer were divided into three groups according to PD-L1 levels in CTCs, High (n=6), Intermediate (n=7) and Low (n=9).
- In our system, we succeeded in the detection of PD-L1 in isolated CTCs. These results showed that PD-L1 expression could be done with the CTC-based platform.
- The establishment of PD-L1 expression based on CytoGen's CTC platform is expected to provide more benefits to the patients who receive anti PD-1 immunotherapy in diverse cancers including bladder cancer.

References

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